IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	LINDA S. POWERS, et al.
SERIAL NO.:	10/706,547
FILED:	November 12, 2003
FOR:	TAXONOMIC IDENTIFICATION OF PATHOGENIC MICROORGANISMS AND THEIR PROTEINS
GROUP ART UNIT:	1641
EXAMINER:	MELANIE J. YU

AFFIDAVIT

STATE OF UTAH)
: ss
COUNTY OF SALT LAKE)

Comes now Christopher R. Lloyd, one of the inventors of the above-entitled invention for the application identified in Serial No. 10/706,547, and responds as follows:

Claim Rejections – 35 USC § 112

6. Claim 26 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The claimed ligand that is a peptide not containing tryptophan or tyrosine, and the detection of such a ligand by intrinsic fluorescence is not disclosed in the specifications. It is noted that the detection of toxins containing tryptophan and tyrosine with tryptophan/tyrosine fluorescence after capture is disclosed in the specifications (pg. 14), but peptides not containing tryptophan or tyrosine are not disclosed.

Response:

The intrinsic fluorescence of proteins, as is known to those skilled in the art, is primarily due to the aliphatic amino acids tryptophan (Trp) and tyrosine (Tyr). These amino acids are excited around 280 nm and emit a relatively broad fluorescence centered about 340–350 nm, this fluorescence is especially strong when these residues are buried inside a hydrophobic protein core (T.E. Creighton, Proteins, 2nd Ed. W.H. Freeman, New York (1993) pp. 14-17). The present application describes using the ***intrinsic fluorescence to detect proteins*** that have been captured by the surface-tethered peptides (claim 26) wherein the surface is excited with light centered around 280 nm and the proteins are detected upon detection of fluorescence emissions in the 340-350 nm region. It would be known to one skilled in the art that if the surface-tethered peptides contained either Trp and/or Tyr then they too would emit this fluorescence upon excitation with 280 nm light. It is the purpose of Claim 26 to detect the captured proteins, not the tethered peptides. Thus, to utilize peptide ligands that contain tryptophan or tyrosine would render the surface fluorescent and incompatible for the intrinsic fluorescence detection of captured proteins.

9. Claim 21, part (b) is vague and indefinite because it is unclear what type of physical separation is utilized. Part (c) is vague and indefinite because it is unclear how interrogation of the substrate surface is performed.

Response:

It is known to those skilled in the art that after capture of the analyte by a surface-associated binding partner that some sort of separation of that surface from the sample is needed to prevent sample components from interfering with

analysis methods; in immunochemical assays this separation is accomplished by (a) removal of the sample from a microtiter well and (b) washing (J. Crowther, ELISA Theory and Practice, **1995**, pp. 35-48, Humana Press, Totowa, NJ) Claim 21, part (b) has been amended to clarify the meaning of physical separation as disclosed in the specification (pp. 11, lines 11-12 and pp. 12, lines 19-20). Claim 21, part (c) has been amended to clarify the role of surface interrogation in analyte identification (pp. 11, line 18) and, in general, how this is to be accomplished.

10. Claim 23, the term "usually" is vague and indefinite because it is a relative terminology. The length of the peptide is unclear because "usually" fails to define a precise range of peptide lengths.

<u>Response</u>:

Claim 23 (and Claims 24 and 25) have been amended to omit "usually," thus defining the ligand as a peptide 3 to 20 amino acid residues in length.

11. Claim 26 is vague and indefinite because it is unclear how a peptide not containing tryptophan or tyrosine and the detection of such peptides are accomplished. It is noted that the detection of a toxin containing tryptophan and tyrosine is disclosed, but this does not provide sufficient disclosure for a peptide ligand not containing tryptophan or tyrosine.

<u>Response</u>:

The intrinsic fluorescence of proteins, as is known to those skilled in the art, is primarily due to the aromatic amino acids tryptophan (Trp) and tyrosine (Tyr). These amino acids are excited around 280 nm and emit a relatively broad fluorescence centered about 340–350 nm, this fluorescence is especially strong

when these residues are buried inside a hydrophobic protein core (T.E. Creighton, Proteins, 2nd Ed. W.H. Freeman, New York (1993) pp. 14-17). The present application describes using ***intrinsic fluorescence to detect protein analytes (not the surface-immobilized peptides)*** that have been captured by the surface-tethered peptides. Detection is accomplished by exciting the surface with ultraviolet light centered around 280 nm and the proteins are detected upon detection of fluorescence emissions in the 340-350 nm region. It would be known to one skilled in the art that if the surface-tethered peptides contained either Trp and/or Tyr then they too would emit this fluorescence upon excitation with 280 nm light. Thus, to utilize peptide ligands that contain tryptophan or tyrosine would render the surface fluorescent and incompatible for the intrinsic fluorescence detection of captured proteins. Claim 26 has been amended to clarify how intrinsic fluorescence is traditionally accomplished.

12. Claim 53 is vague and indefinite because it is unclear whether the specific protein analyte of a proteinaceous toxin or cytosolic protein are part of the claimed invention. The limitation is recited in the preamble, but the body of the claim does not correlate the step of identifying the specific protein.

Response:

Claim 53 has been amended to cite "protein analyte", thus removing the limitation in the preamble but citing the limitation in Claim 53 (d) (amended).

Claim Rejections – 35 USC § 102

14. Claims 21 and 53 are rejected under 35 U.S.C. 102(b) as being anticipated by Hudson et al. (U.S. Patent 5,576,220). Hudson et al. teach a method for identification of a biological analyte comprising: exposing a solution containing

the analyte (Fig. 1, label "TTM"; col. 3, lines 64-67; col. 4, lines 1-5) to a ligand specific for the analyte of interest (Fig. 1, label "L"; col. 3, lines 64-67; col. 4, lines 1-5) that has been covalently tethered (Fig. 1, labels "T", "4", and "6"; col. 5, lines 37-39; col. 4, lines 48-52; col. 14, lines 11-14) to a substrate surface with a photostable linker (Fig. 1, labels "3", "10", "11", "12", and "T"; col. 8, lines 12-24) at a distance of 15-50 Å, which would encompass the recited "at least six Å" (Fig. 1, label "A"; col. 7, lines 48-50) for the capture of proteins (col. 1, lines 27-36; col. 3, lines 14-16); separating the bound analyte from the non-binding components of the solution containing the analyte by physical separation, washing or both (col. 14, lines 36-38; col. 18, lines 5-9); and interrogation of the ligand-tethered surface for analyte binding (col. 1, lines 36-48; col. 3, lines 56-58; col. 14, lines 36-38). Hudson et al. also teach a peptide ligand containing three to twenty amino acids (col. 7, lines 64-67; col. 8, lines 1-11).

Response:

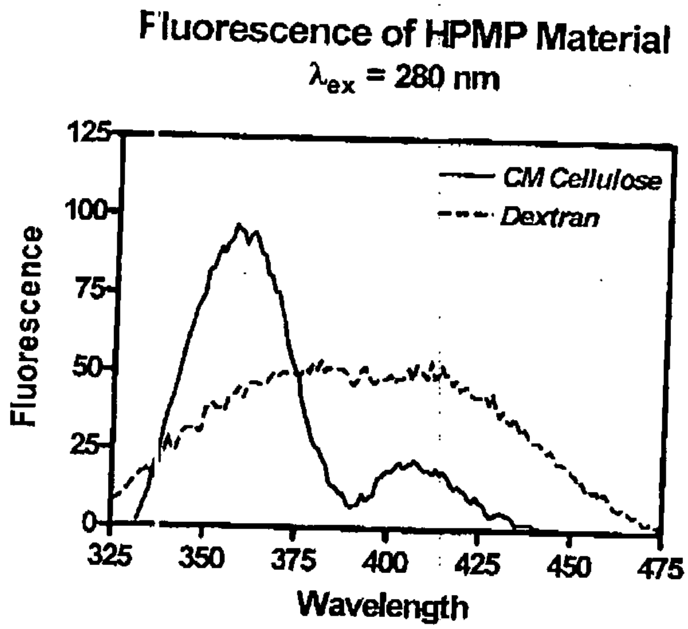
While Hudson et al does teach the binding of analytes (including protein analytes) by tethered peptide ligands, their disclosure differs from the present application in several important ways. The most significant difference between the two is that Hudson describes the binding of "Tagged Target Molecules" (TTMs) by peptide ligands covalently supported on "hydrophilic polar multifunctionalized polymers" (HPMPs) that are tethered to a surface. Hudson et al. teaches that the presence of this HPMP layer (Fig. 1, label "HPMP") is necessary for their invention to: (a) simulate an aqueous environment in order to address ligand solubility issues (col. 3, lines 2-5), (b) target analyte elution considerations (col. 4, lines 63-65), and (c) address detection method

interference concerns (col. 4, lines 65-67 and col. 5, lines 1-5). The present application describes capture of protein analytes (aqueous toxins, hormones and cytosolic proteins) by tethered peptide ligands that are *highly* specific (less susceptible to environmental [pH, ionic strength and temperature] issues; MBS col. 5, lines 31-35) and anchored to a surface with an *appropriately* long photostable tether (MBS col. 4, line 45), removing the unbound material, and finally interrogating the ligand-tethered surface to determine the presence of tightly bound protein analytes (MBS col. 4, lines 50-54). Hudson et al. actually counsels against using surface-tethered peptide ligands (with consequential low aqueous solubility) as close to the surface as the present application teaches due to artifacts introduced by surface effects (col. 3, lines 5-8); this is essential to Hudson et al. since their ligand-analyte bond is expected to be labile (col. 8, lines 12-24; col. 4, lines 48-52), mimic aqueous interactions (abstract) and at times needs to be tethered with cleavable linkers (col. 5, lines 21-24). Peptide ligand to target protein analyte specificity, and the resultant minimal dependence of their binding upon environmental conditions, imparts the ability of the present invention to bind proteins under conditions that cause artifacts for the screening system taught in Hudson et al.

Another important way in which Hudson et al. differs from the present application is that Hudson et al. describes the capture of "Tagged Target Molecules" (Fig. 1, label "TTM"; col. 3, lines 64-67; col. 4, lines 1-5); these TTMs are analytes which have been previously covalently conjugated with labels (col. 8, line 23) by which binding interactions are identified (Example 15, col. 14-15). The present application teaches detection of the bound protein analytes via their

intrinsic fluorescence (claim 26), fluorescence quenching of the ligand (claim 37), and due to the property (fluorescence [claim 26], radioactivity [claim 30], luminescence [claim 32], phosphorescence [claim 34], and optical absorbance [claim 36]) of a conjugate that reacts with the bound analyte after capture.

Hudson et al. teaches a typical spacer arm (tether) of 15–50 Å (col. 7; lines 48-50) which does encompass some of the greater than 6 Å tether lengths disclosed in the current application for the capture of proteins. However, the tether distance described by Hudson et al. is from the substrate surface to the HPMP layer (not the distance from the surface to the ligand). Hudson et al. also teaches (in its preferred method) that these tethers which don't react with the surface can be used to build larger tethers for ligand binding (col. 7, lines 11-13). When combined with the description of the HPMP layer being preferably 1000 Å thick (col. 6, lines 33-37), a covalently linked peptide ligand would be more than 1100 Å away from the substrate surface – this is significantly larger than the 6 Å distance disclosed in the current application. It should also be noted that Hudson et al. teaches that a major purpose of this spacer arm is to control the density of the HPMP anchored to the surface (col. 7, lines 39-40). The tethers described in the current application are used to directly enhance target analyte binding to the tethered peptide ligands.



Hudson et al. also teaches that the HPMP layer must be "non-masking" (col. 4, line 67 through col. 5, lines 1-3), meaning that the HPMP layer must not interfere with the detection of the tag on the analyte. The current application teaches detection of the bound protein analyte via its intrinsic fluorescence. As is known to those skilled in the art, the amino acids primarily responsible for the fluorescent properties of proteins are tryptophan (Trp) and tyrosine (Tyr); Trp and Tyr absorb light at wavelengths around 280 nm and emit light at wavelengths between 340 to 360 nm (U.S. Patent 6,750,006). Figure 1 shows the fluorescence of two polysaccharides examples disclosed in Hudson et al. when excited with light used for intrinsic fluorescence protein detection. Even though neither polysaccharide material contains tryptophan or tyrosine, they fluoresce in a region that would cause interference with intrinsic fluorescence detection.

Thus, to one experienced in determining the intrinsic fluorescence of biological material, it would be clear that HPMP-tethered peptide ligands would not be compatible with intrinsic fluorescence detection of tethered peptide captured proteins.

15. With respect to Claim 53, the specific protein is not given patentable weight since it is recited in the preamble and body of the claim does not correlate the step of identifying the specific protein.

Response:

Claim 53 has been amended to cite "protein analyte", thus removing the limitation in the preamble but citing the limitation in Claim 53 (d) (amended).

Claim Rejections – 35 USC § 103

18. Hudson et al. teach the method of identification of a biological analyte including a peptide ligand containing three to twenty amino acids, as discussed above. Hudson et al., however, do not teach a biological analyte being a proteinaceous toxin, a ligand specific for a proteinaceous toxin, detection of captured analyte through intrinsic fluorescence of the protein, or the identification of a protein analyte wherein the protein is a proteinaceous toxin.

Powers et al. teach a method for taxonomic identification of microorganisms wherein the analyte is a proteinaceous toxin (pg. 1, 1st paragraph and pg. 10, 2nd paragraph) and a ligand specific to a toxin (pgs. 13-14), in order to capture and detect a specific proteinaceous toxin, which are potential infections.

Therefore it would have been obvious to one having ordinary skill in the art at the time the invention was made to include in the method of Hudson et al., a

proteinaceous toxin and a ligand specific to the toxin, as taught by Powers et al., in order to detect and identify proteinaceous toxins within a sample, since these toxins are known as potential infections and also of concern to vertebrate animals including humans, livestock, poultry, and wildlife.

<u>Response</u>:

Hudson et al. describes the capture of "Tagged Target Molecules" (analytes that have already been covalently conjugated with labels) by peptide ligands covalently supported on "hydrophilic polar multifunctionalized polymers" (HPMPs) that are tethered to a surface, relying on the properties of the HPMP to simulate an aqueous environment for the ligand-analyte binding. Though Powers et al. teach a method for taxonomic identification of microorganisms wherein the analyte is a proteinaceous toxin with a ligand specific to a toxin, it teaches neither the steps of physically separating the ligand-coated surface, washing away non-bound portions of the sample (biological components of the matrix from which the analyte was captured) nor the indicated tether lengths that are to be used for each kind of biological analyte. Since Hudson et al. teaches against using surface-tethered peptide ligands (as is taught in the abstract and elsewhere of the PCT publication of Powers, et al.) due to artifacts introduced by surface effects (col. 3, lines 5-8), one having knowledge of Hudson et al. would have been advised against using the method of Powers, et al. to detect proteinaceous toxins.

Additionally, Powers et al. teaches the detection of potential infections of concern to vertebrate animals including humans, livestock, poultry, and wildlife. However, toxins are not themselves infections 'per se' nor infectious (they do not

cause disease); toxins are the causative agent of many symptoms of said diseases.

19. Intrinsic fluorescence, recited in Claim 26, is taught by Powers et al. (pg. 18, 1st paragraph; pg. 32, 1st paragraph).

Response:

Though Powers et al. teach the use of intrinsic fluorescence for the detection of microorganisms and proteins (as do others skilled in the art, see U.S. Patents 5,474,910; 5,760,406; 5,968,766 and 6,750,006) captured by a peptide ligand, it teaches neither the steps of physically separating the ligand-coated surface, washing away non-bound portions of the sample (biological components of the matrix from which the analyte was captured) nor the indicated tether lengths that are to be used for each kind of biological analyte.

Christopher R. Lloyd

Subscribed and sworn to before me this 15 day of November, 2004.

Roberta M. Kelly
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